

# **EFFECTS OF SMELLING NARCOTICS ON SOME BIOCHEMICAL PARAMETERS IN GERMAN SHEPHERD DOGS**

**By**  
**Abdeen Mohammed Abdeen**  
**B.Sc. of Veterinary science 1995**  
**University of Khartoum**

**Supervisor:**  
**Dr. Nabiela Musa El Bagir**

**A Thesis Submitted in Partial Fulfillment for the Requirements of  
the Degree of Master of Biochemistry**

**Department of Biochemistry**  
**Faculty of Veterinary Medicine**  
**University of Khartoum**

September 2005

## **DEDICATION**

To my wife Shadia, my parents, my brothers and sister,  
relative, and to the Department of Biochemistry –  
Faculty of Veterinary Medicine and the Staff of Police  
Dogs Administration.

## **ACKNOWLEDGEMENT**

It is my wish to acknowledge those who , in their own capacity ,have contributed willingly towards achieving my academic objectives .

First I would like to extend my deepest thanks and regards to my supervisor Dr. Nabiela Musa Elbagir , for her interest , close supervision and continuous advice and that she initiated my interest to work in the field of biochemistry .

My thanks also go to Dr. Mohammed Khair – Faculty of animal production – University of Khartoum for his great help in statistical analysis of this work .

I am also very much indebted to the staff in the Department of biochemistry especially Asha the secretary of the Department .

My thanks also go to the staff of the Police Hospital Laboratories for their help.

Especially thanks are for the staff of the Police Dogs Administration , Dr Ali Ahmed Hassan , Khlid , Amar ,Hashim ,Alaldeen , Yasir ,Amani Gusiana for their help and support.

My deep thanks go to my wife Shadia and my family , parents and brothers for their unlimited support.

## Contents

Dedication.....	I
Acknowledgement.....	I
Contents.....	III-IV-V-VI
List of tables.....	VII-VIII
List of figures.....	IX
Abstract.....	X-XI
Arabic abstract.....	XII
Introduction.....	I

## CHAPTER ONE: LITERATURE REVIEW

1.1 Cannabis sativa.....	3
1. 1.1 History.....	3
1.1.2 Source and names of Cannabis.....	3
1.1.3 Uses.....	4
1.1.4 Chemical structure, stability.....	4
1.1.5 Routes of entery.....	5
1.1.5.1 Oral administration.....	5
1.1.5.2 Inhalation.....	6
1.1.6 Metabolism.....	6
1.1.7 Drug effects.....	8
1.1.7.1 Mode of action.....	8
1.1.7.2 Toxicity.....	8
1.1.7.2.1 Human data.....	8
1.1.7.2.2 Animal data.....	8
1.7.3 Carcinogenicity .....	9
1.1.8 Cannabis subjective effects .....	9
1.1.8.1Acute effects.....	9
1.1.8.1.1 Effects and permanent psychosis.....	9
1.1.8.1.2 Adverse effects.....	11

1.1.8.2Chronic ffects.....	11
1.1.8.2.1 ngestion.....	11
1.1.8.2.2 halation.....	12
1.1.9Effects of can nabinoids on some biochemical parameters.....	13
1.1.9.1 Effects of cannabinoids on some plasma enzymes.....	13
1.1.9 .2 Effects of cannabinoids on plasma glucose.....	13
1.1.9.3 Effects of cannabinoids on plasma urea and creatinine .....	15
1.2Heroin.....	15
1.2.1Background.....	15
1.2.2Heroin effects.....	16
1.2.3Heroin long- term effects.....	17
1.2.4 Heroin metabolism .....	18
1.2.5 Effects of heroin on some biochemical parameters.....	18
1.2.5.1 Effects of heroin on some liver enzymes.....	18
1.2.5.2 Effects of heroin on plasma glucose.....	19
1.2.5.3 Effects of heroin on serum creatinine and urea.....	19
1.3 Effect of season on liver enzymes ,urea and creatinine.....	20
1.4 Olfaction.....	20
1.4.1 Structure and function of dog olfactory system .....	21
1.4.2 Comparison of human versus dog.....	22

## CHAPTER TWO: MATERIAL AND METMODS

2-1- Experimental animals.....	25
2-2- Housing.....	27
2-3- The routine training program.....	27
2-4- Samples collection.....	28
2-5- Biochemical methods.....	28
2-5-1- Determination of plasma alkaline phosphatase (ALP).....	28
2-5-2-Determination of plasma glutamic oxalo acetic transaminase.....	29
2-5-3- Determination of glutamic pyruvic transaminase (GPT).....	30
2-5-4-Determination of glucose.....	31
2-5-5- Determination of urea.....	31
2-5-6- Determination of Creatinine.....	32

---

## CHAPTER THREE : RESULTS

3.1The effects of narcotic detection by smelling on some biochemical parameters in German shepherd dogs.....	35	
3.1.1		liver
enzymes.....	35	
3.2.1.Plasma	urea	and
creatinine.....	35	
3.1.3		Plasma
glucose.....	37	
3.2 The effects of sex , age and season on plasma parameters of narcotic detectors.....	37	
3.2.1 Plasma glucose in narcotic detectors dogs.....	37	
3.2.2 Plasma creatinine.....	39	
3.2.3.Plasma urea.....	40	
3.2.4 Plasma alkaline phosphatase ( ALP ) .....	42	
3.2.5 Plasma glutamic oxalo acetic transaminase ( GOT).....	43	
3.2.6 Plasma glutamic pyruvic transaminase ( GPT ).....	45	

## CHAPTER FOUR DISCUSSION

4.1 The effects of narcotic detection by smelling on some biochemical parameters in German shepherd dogs.....	47
4.1.1 liver enzymes.....	47
4.1.2 Plasma urea and creatinine .....	48
4.1.3 Plasma glucose.....	49
4.2 The effect of sex , age and season on plasma parameters of narcotic detectors.....	50
4.2.1 Plasma creatinine .....	50
4.2.2 Plasma urea.....	51
4.2.3 Plasma alkaline phosphatase (ALP).....	51
4.2.4 Plasma glutamic oxaloacetic transaminase (GOT).....	51
4.2.5 Plasma glutamic pyruvic transaminase ( GPT ) .....	52
4.3 Conclusions.....	53
References.....	54

### List of tables

Table (1):Vaccination program of experimental dogs.....	25
Table (2) :Deworming program of experimental dogs.....	26
Table (3) : The effects of narcotic detection by smelling on different biochemical parameters in German shepherd dogs .....	36
Table (4) : Effects of treatment and sex on plasma glucose in German shepherd dogs .....	37
Table(5) : Effects of treatment and age on plasma glucose in German shepherd dogs.....	38

Table (6) : Effects of treatment and season on plasma glucose in German shepherd dogs .....	38
Table( 7) : Effects of treatment and sex on plasma creatinine in German shepherd dogs .....	39
Table (8) : Effects of treatment and age on plasma creatinine in German shepherd dogs .....	40
Table (9) : Effects of treatment and season on plasma creatinine in German shepherd dogs .....	40
Table (10) : Effects of treatment and sex on plasma urea in German shepherd dogs .....	41
Table (11) : Effects of treatment and age on plasma urea in German shepherd dogs.....	41
Table (12) : Effects of treatment and season on plasma urea in German shepherd dogs .....	42
Table (13) : Effects of treatment and sex on plasma alkaline phosphatase (ALP) in German shepherd dogs.....	42
Table (14) : Effects of treatment and age on plasma alkaline phosphates (ALP) in German shepherd dogs.....	43
Table(15) : Effects of treatment and season on plasma alkaline phosphatase (ALP)in German shepherd dogs.....	43



Table (16) : Effects of treatment and sex on plasma glutamic oxaloacetic transaminase (GOT ) in German shepherd dogs .	44
Table (17) : Effects of treatment and age on plasma glutamic oxaloacetic transaminase (GOT ) in German shepherd dogs.....	44
Table (18) : Effects of treatment and season on plasma glutamic oxaloacetic transaminase (GOT ) in German shepherd dogs	45
Table (19) : Effects of treatment and sex on plasma glutamic pyruvic transaminase (GPT ) in German shepherd dogs	45
Table ( 20) : Effects of treatment and age on plasma glutamic pyruvic transaminase (GPT ) in German shepherd dogs.....	46
Table (21) : Effects of treatment and season on. plasma glutamic pyruvic transaminase (GPT ) in German shepherd dogs.....	46

### **List of figures**

Fig No (1) : Crude heroin.....	24
Fig No (2) : German Shepherd dog during searching for C-sativa .....	33
Fig No (3) : German Shepherd dog found the C-sativa and holding it ..	34

## **Abstract**

This work was conducted in Biochemistry Department Faculty of Vet. Medicine University of Khartoum to study the effects of smelling narcotics (C.sativa and heroin) on some biochemical parameters in German shepherd dogs.

The study targeted Police German shepherd dogs in Police Dogs Administration. Twenty-six adult German shepherd dogs, males and females 2-9years old , weighing between 25-35kg,working in the Police Dogs Administration, were selected, divided as 10 dogs as C.sativa detectors, 6 dogs as heroin detectors and other 10 dogs as control group . The blood samples were taken from the cevalic vein for investigation of SGOT ,SGPT, ALP, glucose, urea and creatinine in three different seasons summer, winter and autumn . The effects of sex , age and season were considered for all parameters .

The results obtained showed that, the mean values of plasma alkaline phosphatase and glutamic pyruvate transaminase for the C. sativa detectors were at similar levels compared to the control group, whereas the mean values of glutamic oxaloacetic transaminase (GOT) were slightly higher compared to the control. Heroin detectors showed similar level for all enzymes compared to control. The mean values of plasma urea and creatinine for C.sativa detectors showed slight increase compared to the control but the difference was not significant. The same effect was also observed in the heroin detectors.

Plasma glucose showed slight decrease for C.sativa detectors, but there was no effect observed for the heroin detectors. No difference was observed between males and females, young and old dogs for all parameters.

All parameters, except glucose, showed significant increase ( $p < 0.05$ ) in summer and autumn compared to the levels measured in winter for both treated and un treated animals.

### خلاصة الأطروحة

أجريت هذه الدراسة بقسم الكيمياء الحيوية بكلية الطب البيطري بجامعة الخرطوم لمعرفة تأثير الكشف عن المخدرات عن طريق الشم في الكلاب الشرطة من فصيلة كلب الراعي الألماني على بعض القياسات البيوكيميائية .

تم اختيار (26) كلبا شرطيا ذكورا وإناثا ، تتراوح أعمارها ما بين 2-9 سنوات ، وأوزانها من 25-35 كيلو جرام في إدارة الكلاب الشرطة ، قسمت إلى ثلاث مجموعات (10) كلاب كلاب لم تعرض للمخدرات كمجموعة للحشيش (البنقو) ، (6) كلاب كمجموعة هيروين و (10) كمجموعة ضبط نتائج . تم أخذ عينات الدم من الوريد الساعدي لقياس الجلوكوز في الدم ، بعض و قياسات لوظائف الكلى مثل اليوريا و ALP و SGPT ، SGOT أنزيمات الكبد تمثلت في الكرياتينين في ثلاثة فصول مختلفة من العام مع أخذ العمر و الجنس في الاعتبار . النتائج التي تم الحصول عليها كانت كالآتي :-

لم تتغير معدلاتها لمجموعة البنقو مقارنة بمجموعة الضبط ALP و 1GPT) أنزيمات أل أظهر ارتفاعا طفيفا . GOT بينما إنزيم أل

(2) كل الانزيمات السابقة لم تتغير بالنسبة لمجموعة الهيروين مقارنة بمجموعة الضبط . (3) معدلات اليوريا و الكرياتينين أظهرت ارتفاعا طفيفا لمجموعة البنقو و الهيروين مقارنة بمجموعة الضبط لكنه غير معنوي .

معدل جلوكوز الدم أظهر انخفاضا طفيفا و غير معنوي لمجموعة البنقو مقارنة بمجموعة (4) الضبط لكنه لم يتغير لمجموعة الهيروين.

لم يكن هنالك تأثيرا واضحا للجنس و العمر (5)

كل القياسات لانزيمات الكبد و اليوريا و الكرياتينين في كل المجموعات أظهرت ارتفاعا (6) معنويا في الصيف و الخريف مقارنة بالشتاء .

## INTRODUCTION

Training of police dogs as narcotics detectors, to look for drugs like heroin, cocaine and hashish (*Cannabis sativa*), is a well known practice carried out world wide. Many studies were made on these dogs to improve their talent , also many studies were made on the drugs to investigate their effects on the different body systems by using different routes of administration , on experimental animals and consequently on humans. These studies showed direct effects of these drugs on different body systems , Huy *et al*,.(1975 ) , Cohen, *et al*; (1971) , Ghoneim ,*et al*;(1980) , Mahfouz *et al* ,(1975) and Hussein , (2003) reported that *C. sativa* use lead to various degrees of liver dysfunction . Some authors reported that hashish lead to some degree of renal dysfunction Ghoneim, *et al* ;(1980 ) and Hay , *et al* ; (1975).

Heroin remains the most common frequently abused narcotic drug especially when crude processing methods are used to manufacture it (McCoy,1991 ) and its use has long been associated with crime because its importation and distribution are illegal and because many addicted people turn to theft and prostitution to obtain money to buy the drug. Also many studies mentioned that heroin lead to serious liver and renal dysfunction in humans and experimental animals Blevins, (1976) (Malik,*et al*; 1992) , (Pearce, 1980) and (Darke, *et al*;1996 ).

German shepherd dogs are at very sensitive olfactory system. These dogs have got an ability to scent 44 times greater than humans since human receptor site contains approximately 5 million cells whereas the German Shepherd dog has approximately 220 million cells ( Steve and Tim,1994).

In Sudan, police use German shepherd dogs as (Bango) detector since (1967) and as heroin detector since (1997).

The present work is designed to investigate the effect of narcotics smelling ,on dogs (German Shepherd) trained to look for and fetch drugs namely Cannabis sativa and heroin.

This is by collecting blood samples to measure the activity of some liver enzymes ( SGOT, SGPT and ALP) and the levels of some important biochemical parameters , i.e. creatinine , urea and blood glucose .All these parameters were known previously to be affected in drugs users Sanz *et al* , (1985) .

These investigations will be carried during summer, winter and the rainy season , so as to evaluate the effect of the season on these animals. Other group of dogs, which are never exposed , to drugs will be used as control and compared to the narcotics detectors group.

## **CHAPTER ONE**

### **LITERATURE REVIEW**

#### **1.1 Cannabis sativa**

##### **1. 1.1 History**

Cannabis sativa use has been reported as early as human race, it was the most widely spread and commonly used illicit drug .The number of Cannabis users world wide were found to be 141 Million(UNDCP,1997). The high risk circumstances of use and abuse vary according to the country or region and the highest rate was reported in developed countries .The use of herbs shoot bark leaves and plant is to relieve pains and help to control disease, since the earliest time had been mentioned. Chinese ,Indian ,Egyptian and Arab used Cannabis plant as medicinal herb to control disease, as anesthetic ,antiseptic and applied externally as analgesic (Kabelik,*et al*;1960 , Ellen and Jonathan ,1973, Sachindra 1977, Mechoulam and Lander ,1980 ).

##### **1.1.2 Source and names of Cannabis**

All plants of genus Cannabis belong to one species Cannabis sativa with two species of sativa (fiber types) and indica (drug types) and with wild and cultivated varieties (Small and Cronquist, 1976) .

Cannabis plant exists in both male and female forms ,it is prepared by drying the leaves and flowering the hemp plant, where the highest concentration of cannabinoids are found cut ,dried ,chopped and incorporated into cigarette (Van Sickle, 1969 ,George and John, 1973 ). Scientific name Cannabis Sativa L. (var Indica),and many local names used Cañamo de la India (Latin America),Chanvre,Herbe (France),

Hanfkrout (Germany), Indian hemp (USA, UK), Marijuana (USA, UK) Hashish (Egypt, Lebanon) and between harvest and use Street marihuana concentrations, called "sin semilla" (seedless) has usually higher of delta-9-THC of 7 - 14% and Banjo in (Sudan).

### **1.1.3 uses**

Medicinal delta-9-THC and some synthetic analogues are used therapeutically, e.g. for nausea and vomiting produced by anti neoplastic chemotherapy. Synthetic cannabinoids used therapeutically include "Dronabinol" nabilone and "Levonamtradol". A further possible indication is to reduce intra-ocular pressure in the treatment of open angle glaucoma. Some synthetic cannabinoids are undergoing clinical trial as analgesics and anticonvulsants. Cannabis sativa has been mixed with other plants in the preparation of anti-asthma cigarettes. Medical uses have been limited by adverse effects similar to those observed after smoking marihuana, but Dronabinol and nabilone have been approved by regulatory authorities in the United States for therapeutic use (Reynolds, 1982).

**Abuse:** marihuana is the most frequently abused drug in the world after alcohol and tobacco and it is the main illegal drug of abuse. From religious point of view some cultures accept its use for a defined religious purpose (e.g. some Buddhist and Tibetan sects, and groups in the north of Africa) (Varma, *etal* ;1988) .

### **1.1.4 chemical structure, stability:-**

CAS number = 1972-08-3

Molecular weight = 172

**Solubility:**

Practically insoluble in water, soluble 1 in 1 in alcohol and ketenes and 1 in 3 of glycerol. It is soluble in fixed oils.

**Stability:**

decreases with time, specially in the case of hashish, but may resist high temperatures without inactivation.

The concentration of delta-9-THC in a sample depends upon the genetic structure of the plant, local conditions of growth, storage methods and time elapsed.

Hashish, the oily dark substance obtained after dissolving the marihuana paste contains 20 to 30% of delta-9-THC. One drop induces an effect similar to one cigarette. It is generally commercialized as fragments of solid resin called "chocolate (Reynolds, 1982 and Jaffe, 1986) .

**Other physico-chemical characteristics**

It has a penetrating, sweetish aroma, very persistent that impregnates clothes and hair of users. Cannabinoids are soluble in alcohol, ether and other organic solvents, but non-soluble in water and mineral acids.

Indian hemp contains more than 60 cannabinoids including: cannabinol, cannabidiol, cannabinolic acid, cannabigerol, cannabicyclol, and various tetrahydrocannabinol isomers, the most important is delta-9-tetra hydro cannabinol (Hardin and Arena, 1974).

**1.1.5 ROUTES OF ENTRY****1.1.5.1 Oral administration**

Not common in cases of abuse but it is the usual route of administration for medical purposes. Absorption from the gastrointestinal



tract is almost complete . Peak blood levels and maximal pharmacological effects occur later after oral administration than after inhalation (Cone,*etal*; 1988). Symptoms become apparent within 30 - 120 minutes reaching a peak after 2 - 3 hours (Schwartz, 1987; Jaffe, 1986).

#### **1.1.5.2 Inhalation**

The commonest way of consuming marihuana and hashish. The inhaled smoke of one cigarette ("joint") contains 0.5-0.7 g delta-9THC. Marihuana can be smoked directly or through small pipes or "bongs", they are similar to those used with opium, where refrigeration by air or water reduces the irritative effects on the trachea-bronchial tract allowing a deeper and prolonged inhalation. The usual technique consists in inhaling very deeply and maintaining the smoke in the lungs for 20 or 30 seconds to maximize the absorption of cannabinoids; the extraction is about 50% of the cannabinoids content. . After inhalation, peak plasma concentrations are achieved within a 7 - 10 minutes; subjective effects appear in 20 or 30 minutes but rarely persist for more than 2 - 3 hours (Jaffe, 1986).

#### **1.1.6 Metabolism**

After oral administration but not after inhalation, delta-9- THC undergoes first-pass hepatic metabolism via enzymatic hydroxylation and carboxylation to the active metabolite 11- hydroxy- delta-9-THC, then carboxylation to the more polar inactive metabolite, 11-nordelta-9-THC acid. Enter hepatic circulation occurs and only 35% is excreted in the urine. Around 80 cannabinoid metabolites can be identified from a

similar metabolic pathway , the most important one is 11- hydroxy-delta-9-THC which is metabolized to non- cannabinoid metabolites such as terpenes and alkenes. Delta-9-THC and its metabolites persist in human plasma for several days or weeks (Jaffe, 1986) . But repetitive ingestion or smoking over weeks is not followed by clinically apparent accumulation , this suggests that the persistent metabolites are inactive. Chronic marihuana smokers metabolize delta-9-THC more rapidly than non-smokers. Metabolites accumulate in tissues with repeated cannabinoid exposure , the biological significances of which were unknown ,the plasma half life being 20-30 hours (Lemberger, *etal*;1976, Kephalis, *et al* ;1976 and Wall,*et al*; 1981 ).

Delta 9-THC is metabolized rapidly due to the plasma concentrations of delta-9-THC and 11- hydroxydelta redistribution in the fatty tissues, afterwards there is a slow decline with a half-life of 30 hours due to the metabolism and the half-life maybe increased in chronic users to 4.1 days (Johansson, 1988).

Delta-9-THC is metabolized by the liver. It is intensively lipophilic and high concentrations accumulate in fatty tissues in great quantities, these are liberated slowly into the circulation (Jaffe, 1986). The major urinary metabolites are carboxy –THC and polyhydroxy acid which are also found in feces ,the major route of excretion , where 11-OH- THC is also the major component but lipophilic THC is not excreted by the kidneys(Ohlsson, *etal*;1982).

Rapid THC clearance occurs from the blood to various tissues such as lungs ,heart ,salivary glands ,spleen ,adrenals, fat ,brain, liver ,kidneys and reproductive tissues with chronic doses resulting in fat and liver. Whereas cannabinoids affect the CNS as well as endocrine system and reproductive system . In fact there is no system in the body that

cannabinoids dose not affect. Cannabinoids work at the cellular level by modifying the permeability of the cell walls, and Delta9-THC and metabolites half life of approximately 72 hours, thus, these products have long range consequences (Nahas, *et al*;1981 and Carlton , 1983 ) .

### **1.1.7 Drug effects**

#### **1.1.7.1 Mode of action**

No specific mechanism or site of action has been demonstrated , the effects on the CNS can be determined by a diminution of cholinergic activity at neuronal level. Psychological effects do not depend on dopaminergic or noradrenergic action. The cardiovascular effects are (tachycardia, decubitus systolic and orthostatic hypotension)counteracted by propranolol (Jaffe, 1986).

#### **1.1.7.2 Toxicity**

##### **1.1.7.2.1 Human data**

In adults the average toxic dose is 0.035 mg/kg body weight (Schwartz,1987) . The minimal effective dose of delta-9-THC is 5 mg. A 0.5 -1 g marihuana cigarette contains 0.5 - 11% delta-9-THC (Jaffe, 1986). Assuming that the average concentration is 5% delta-9-THC and that 50% is destroyed by pyrolysis during smoking, the total inhaled dose is approximately 25 mg of this, approximately 60% is absorbed by inhalation. (Nahas ,1975) estimated that the lethal dose by intravenous injection is 2 g for a 70 kg person. The minimum plasma concentration of delta-9-THC which produces psychotropic effects is 25 ng/ml (Hollister, 1988).In children no data is available.

##### **1.1.7.2.2 Animal data**

With Cannabis extract, the LD50 in mice is oral 21.6 g/kg ,dermal 11g/kg and IV 0.18g/kg body weight (Valbuena , 1987).

### **1.7.3 Carcinogenicity**

Marihuana smoking and hashish abuse produce histological changes and affect the bronchial epithelium in the young animal. In animals, the tar produced by marihuana pyrolysis is more carcinogenic than that of tobacco (Jaffe, 1986). High temperature burning corresponding with deep inhalation into the lungs, together with smoking until the end of the cigarette, all increase the carcinogenic risk of delta-9-THC and polyaromatic hydrocarbons.

### **1.1.8 Cannabis subjective effects**

#### **1.1.8.1 Acute effects**

The acute effects of cannabis depend on the concentration of delta-9-THC in plasma, the concentration of delta-9-THC in the marihuana cigarette, the inhalation technique (prolonged and deep inhalation; use of bong, pipes, others), individual and environmental conditions. Individual conditions depending upon the person, such as previous experiences, attitude, expectations for the actual experience, and personality. In this way, inexperienced young persons, fearing to be discovered, can present acute anxiety reactions and panic fear of losing self-control and unpleasant sensations. Young people with unstable personalities and acute affective disorders, such as depression or a psychotic background (unrelated to drugs), have a higher risk of developing adverse (Cohen, 1982).

#### **1.1.8.1.1 Effects and permanent psychosis**

Environment Conditions due to the environment (confidence in partners, link between users and other participants comfort, safety, etc). Interactions between cannabis and other drugs of abuse and alcohol may provoke unpredictable effects in individuals and the response to delta-9-THC may be very complex.

The usual clinical picture appears after smoking a cigarette with 2% of THC, or after a 20 mg dose of delta-9-THC. After a few minutes, the first effects on humor, motor coordination, sensitivity, auto-perception, cognitive capacity attention and time perception occur. Feelings of well-being, euphoria, laughing and relaxation are common, somnolence is observed when the individual is alone. Tasks that require intermediate steps before reaching the objective are difficult to perform (time misperception). There is a tendency to mix past, present and future with a strange feeling of unreality and depersonalization. Motor coordination (balance and reaction time) is always affected, even at low doses, therefore a higher risk of accidents, perception of color, distance, and depth, and visual acuity is impaired. These effects are more persistent than the subjective disorders, and they last for 4 - 24 hours.

Marihuana smokers frequently experience hunger, dryness of the mouth and throat, vivid visual images, hyperacusia, and increased sensations of touch, taste and smell. There is a reduction of empathy and perception of other people's emotions, conversation may be unclear and communication may be interrupted by unrelated words and ideas.

At higher doses the patient may have: hallucinations, delirium, paranoia and variable degrees of anxiety culminating in panic and toxic psychosis. There is an increase in heart rate, with high supine systolic blood pressure and orthostatic hypotension. Conjunctival irritation is usually seen.

The patient's body temperature is increased by inhibition of sweating. Respiratory effects are related to the chronic use of marihuana, the acute effect is bronchodilatation both in healthy and asthmatic individuals. But in the latter, irritative effects may precipitate an asthmatic crisis.

A painful, itching or burning sensation of mouth and throat produces irritating cough. Hashish smokers develop inflammation and swelling of the eyes. The cannabis "trip" may be interrupted easily voluntarily, so that the person may look normal, even in his effective relations and in the speed and contents of speech. After 2 or 3 hours, the user may gradually "leave" the intoxicated state and develop clumsiness (physical and mental), irritability (that may turn into rage), somnolence and deep sleep. Depression may occur. During this "coming down" phase, the avidity for food rich in carbohydrates, sweets and cola soft drinks is common. Once the "trip" is over, there is practically no hangover. Only two physical signs persist: tachycardia and conjunctival irritation (although the latter can be avoided by use of eye drops). Although the effects of marihuana are usually pleasant, adverse effects may be observed even when the consumer is experienced, and may be seen also after a single dose, even if it is low (Zuckerman, *etal*;1989).

#### **1.1.8.1.2 Adverse effects**

Acute toxic psychosis with: excitation, confusion disorientation illusions, depersonalization, visual hallucinations and delirium, acute panic reactions ("bad trip") accompanied by abdominal pain, headaches, anxiety, depression with excessive fear of being discovered, fear of dying and uncontrollable aggressive feelings with paranoid ideas. "flashback" reactions are not frequently associated with cannabis but some cases have been reported (Schwartz, 1987).

### **1.1.8.2 Chronic effects**

#### **1.1.8.2.1 Ingestion**

No data is available.

#### **1.1.8.2 .2 Inhalation**

The chronic effects of cannabis are controversial, but believed to be more important, the younger the patient starts with the abuse. They may consist of a motivational syndrome although marijuana may be a primary or secondary factor inducing a motivational syndrome, it is not the main one, a drug that produces passiveness is effective only in the predisposed individual (Cohen, 1982). The syndrome consists of: loss of interest, apathy, passiveness lack of interest in work and productivity without any concern lassitude and loss of energy, lack of tolerance and easy frustration melancholy, bad temper and whims loss of concentration and inability to process any new information may lead to abuse of other drugs, a phenomenon that has been called "escalation crime: chronic abusers may be involved more frequently in illegal activities. Tolerance and dependence are mainly due to functional or pharmacodynamic adaptations of the CNS, rather than to faster metabolism and excretion. Tolerance develops to emotional changes, tachycardia, body temperature and psychomotor tasks, tolerance of the cardiac effects may develop in just a few days. After chronic abuse at high doses, sudden discontinuation produces: irritability, restlessness, nervousness, loss of weight, insomnia, tremors, rise of body temperature and shivering. Symptoms may start a few hours after withdrawal, and last a few days (Schwartz, 1987 ; Jaffe, 1986) .

### **1.1.9 Effects of cannabinoids on some biochemical parameters**

#### **1.1.9.1 Effect of cannabinoids on some plasma enzymes**

Huy, *et al.*, (1975) observed in guinea-pigs given dose of delta9-THC for six months at rate of five injection/week, toxic effects of the drug were caused by its accumulation in the liver, which provoked an inhibition of certain liver enzymes used to digest the hepatic glycogen..

Cohen, *et al.*; (1971), Patton and Pertwee, (1972) showed that a single dose of delta 9-THC succeeds in inhibiting hepatic microsomal enzymes. Ghoneim, *et al.*; (1980) exposed rabbits to hashish smoke every other day for one month resulted in inhibition on incorporation of amino acids into protein, resulted in a significant increase in glutamate dehydrogenase Activity, an enzyme which is primarily involved in ammonia formation from amino acids. Serum glutamate oxaloacetate transaminase (GOT) activity was increased significantly in adult male rats exposed to hashish smoke for 15 min, whereas serum glutamate pyruvate transaminase (GPT) was unaffected. This assumption is based on the fact that (SGPT) which is reported by many authors to be a more sensitive measure in evaluating hepatocellular damage than (SGOT). Furthermore trauma of skeletal muscle is one of the factors known to increase (SGOT) activity. On the other hand, it is possible that if (SGPT) was measured after a longer period of time, a rise of this enzyme might have been detected. Hashish and its constituents have been affected certain parameters in the liver, such as changing the level of certain hepatic enzymes and causing certain degree of liver dysfunction (Mahfouz, *et al.*; 1975).

#### **1.1.9.2 Effects of cannabinoids on plasma glucose**

Pemutt, *etal.*; (1976) observed the effect of marihuana on carbohydrate metabolism in fed and fasting state in chronic marihuana users. They



did not record hypoglycemia in 7 patients who were given marihuana after fasting for 24-72 hours .They also found no significant differences in carbohydrate tolerance and no hypoglycemia during an oral glucose tolerance test in 10 patients who smoked marihuana on alternate days .Also marihuana has no effect on carbohydrate metabolism in the fed or fasted in well nourished chronic marihuana abuse .In ganja users glucose tolerance was decreased indicating modified carbohydrate metabolism , which may aggravate a diabetic condition or expose a latent diabetic individual. This was expressed as complaint of weight loss with increased appetite , nausea ,abdominal pain , dry mouth and excessive frequent urination . THC decreased glucose utilization by cells with corresponding decrease in lactate excretion , (Madelaine , 1985 ).

When Mahfouz, *et al*;(1975 ) exposed adult rats to hashish smoke for 15 minutes ,they observed a significant increase in blood glucose level 24% as compared to the control value . One possible explanation could be that exposure to hashish smoke increases the level of adrenaline in the blood , adrenaline is well known to cause an increase in the blood glucose .

When adult rats were exposed to acute intoxication of cannabis (600 mg / kg Bwt dose within 36 hours of cannabis extract in olive oil) it was observed that energetic and detoxifying metabolism of glucose increased parallel to the observed decrease of glycogen (Sanz ,*etal* ;1985 ) .The administration of guinea pig with acute dose of delta 9-THC for six months of five injection / week , showed that THC produced no change on serum glucose (Huy, *et al*;1975 ).

When rats injected with high doses (0.6 mg /gm Bwt) for ten days with four doses ( day 1 , 4 , 7 and day 10 ) showed no significant decrease following the last dose (Hussein ,2003).

### **1.1.9.3 Effect of cannabinoids on plasma urea and creatinine**

Exposure of rabbits to hashish smoke every other day for a period of one month resulted in marked increase in blood ammonia, and increased blood urea concentration in these animals. Possibility for the raised blood levels, would be the assumption that there is an increased rate of amino acids breakdown in these animals leading to the formation of a large amount of ammonia, to stimulate the activity of enzymes involved in urea biosynthesis and increased urea level and rhabdomyolysis increase plasma creatinine, (Ghoneim, *et al*;1980 ).

THC produced no changes on serum urea nitrogen in guinea pig treated with delta 9- THC at the rate of 3 mg / kg Bwt daily for six months (Huy, *etal*;1975).

## **1.2 Heroin**

### **1.2.1 Background**

Heroin (diacetylmorphine) is a semi synthetic narcotic that was first synthesized in (1874 ). It was originally marketed as a safer, nonaddictive substitute to morphine. Soon after introduction, it became clear that heroin was as addictive as morphine. The USA prompting government to institute measures to control its use. By (1914 ), the ( Harrison Narcotics Act ) prohibited the use of heroin without a prescription. In ( 1920 ), the (Dangerous Drugs Act ) prohibited the use of heroin altogether. Heroin is a white powder with a bitter taste. Street heroin samples are frequently mixed with other substances so that dealers may maximize their profits. Because of these impurities and colors, additives, street heroin may appear in a variety of hues and ranging from white to dark brown (Booth,1996 ) . Heroin is occasionally sold as a black tarry substance,

underground in the United States, frequently abused narcotic, followed by codeine heroin remains the most common drug especially when crude processing methods are used to manufacture it (McCoy,1991 ).The presence of impurities and additives also limit heroin absorption through mucous membranes, thus limiting its "rush" and "high" when sniffed or snorted. For the dependent patient, intravenous injection ("mainlining") becomes the only effective method of heroin abuse. Recently, the purity of street heroin has increased significantly, and its price has dropped sharply. In (1980 ), for example, the average street sample (100-mg bag) contained 3.6%heroin (3.6 mg of heroin) and costs \$3.90, compared to (1999) when the average sample contained 38.2%heroin and cost \$0.80. Samples from South America appear to have the highest purity, reaching the 90% range. Not surprisingly, this dramatic increase in heroin purity, coupled with the well-publicized dangers of intravenous drug use, has led to a change in the pattern of use. Snorting and smoking are slowly becoming the methods of choice and are especially favored by the younger population and new users. In (1997) the National Household Survey on Drug Abuse (NHSDA) survey estimated the lifetime prevalence of heroin use at 2.0 million, the number of current users at 325,000, and the number of new abusers at 81,000. Nearly 100% of new heroin abusers are younger than 26 years. The true prevalence of heroin use is probably much higher than reported, because surveys do not reach all places (David ,1987).

### **1.2.2 Heroin effects**

The short-term immediately effects are:

- One-to-two minute "rush."
- Warm flushing of the skin.
- Dry mouth.

- Heavy feeling in arms and legs.
- Nausea.
- Vomiting.
- Severe itching.
- Slowed heart rate
- Slowed breathing rate.
- Suppression of pain.
- Reduced anxiety.
- Reduced coughing .
- "Nodding" or alternating between a wakeful and drowsy state .
- The pupils of the eyes become smaller.
- Drowsiness and confusion for up to six hours (Darke, 1996).

### **1.2.3 Heroin long - term effects :**

**1.2.3.1 Tolerance:** more and more drug is needed to produce the euphoria and other effects on behavior.

**1.2.3.2 Addiction:** psychological and physiological need for heroin. People are driven to get more heroin and feel bad if they do not get it. People begin to crave heroin 4 to 6 hours after their last injection (Darke,1996) .

**1.2.3.3 Withdrawal:** About 8-12 hours after their last heroin dose, addicts' eyes tear, they yawn and feel anxious and irritable. Excessive sweating, fever, stomach and muscle cramps, diarrhea and chills can follow several hours later. These withdrawal symptoms can continue for 1 to 3 days after the last dose and can last 7 to 10 days. In some cases, full recovery can take even longer.

#### **1.2.4 Heroin metabolism**

Heroin has a high lipid solubility and rapid penetration to the brain. The half-life of heroin is 15-30 minutes. It is rapidly converted to 6-mono-acetyl-morphine (6-MAM) by the liver, brain, heart, and kidney and may not be detected in the blood at the time of blood draw. 6-MAM is then converted to morphine. Morphine is metabolized by the liver and excreted as a glucuronide product or in its free form by the kidneys. Morphine's half-life is considerably longer than heroin on the order of 2-3 hours. A small amount of 6-MAM is excreted in the urine unchanged for up to 24 hours after heroin use ( Washton ,1981 ).

#### **1.2.5 Effects of heroin on some biochemical parameters**

##### **1.2.5.1 Effects of heroin on some liver enzymes**

The liver is the major organ in the body for detoxification. Heroin abuse leads to toxicity in the body and therefore the liver is vital in ridding the body of many toxic substances. Also heroin use may have resulted in impaired liver function (Pearce and Cox, 1980) .Heroin causes many toxic effects on livers of addict people. (Malik, *et al*; 1992 and Belvins, 1976).

Heroin was found to change the expression of cytochrome P450 and other activities of carcinogen-metabolizing enzymes in the liver of male mice (Sheweita,2003) .

. When rats injected with heroin 20 mg weekly for three weeks, serum alkaline phosphatase (ALP), glutamic oxalo acetic transamines (GOT) and glutamic pyruvate transamines (GPT) increased significantly, then returned to the normal level after 14 days (Baltimore ,1993 ). Heroin addicts showed high levels of (GOT), (GPT) and alkaline phosphatase (ALP) (Gruskin, 1999).

#### **1.2.5.2 Effects of heroin on plasma glucose**

Heroin addiction seems to produce a beta-cell failure and contemporaneously a state of hyperinsulinaemia. It is concluded that chronic heroin addiction may produce a change in the rate of hepatic extraction of insulin. In heroin addicts the glycemic response to the glucose load shows a delayed peak time. The insulin curves show increased insulin peaks, delayed peak time and prolonged hyperinsulinemia and significant decrease in plasma glucose (Foltin,etal; 1986).

#### **1.2.5.3 Effects of heroin on serum creatinine and urea**

Heroin toxicity causes high level of serum creatinine and urea . Heroin over dose causes acute renal failure and rhabdomyolysis in addict people (Ellenhorn,*etal*;1999). Rhabdomyolysis literally means the breakdown of striated muscle fibers that results in the release of muscle cell contents into the blood .Heroin induced is a potent vasoconstrictor, causes rhabdomyolysis by limiting the blood supply to the muscle.

Rhabdomyolysis leads to acute tubular necrosis (ATN) and its a potentially reversible renal failure. During the course of acute renal failure, waste products such as urea , nitrogen and creatinine accumulate in the blood resulting in high levels of serum creatinine and urea (Darke, *et al* ;1996 ).

### **1.3 The effects of season on liver enzymes ,urea and creatinine**

Complication of heat stress in dogs including central nervous damage. Hepatic injury and rhabdomyolysis with renal injury and cardiac abnormalities. Rhabdomyolysis due to hyperthermia lead to high level of plasma urea and creatinine and hepatic injury increases plasma alkaline

phosphatase , ,glutamic oxalo acetic transaminase (GOT) , glutamic pyruvate transaminase (GPT) (Clowes and O'Donnell ,*etal* , 1974 ).

Heat stress can lead to serious damage to the kidney , liver ,heart and lungs of German shepherd dogs .Liver damage increased serum liver enzymes caused thrombocytopenia, increased plasma fibrin degradation , elevations in glutamic pyruvate transaminase (GPT) levels due to liver injury and decreases in platelets number (Knochel and Reed, 1987).

In dogs hyperthermia may lead to muscles damage, rhabdomyolysis and hepato cellular damage that elevates serum enzymes such as serum (GOT) , (GPT) and alkaline phosphatase (Sawka, 1985 ) .

#### **1.4 Olfaction**

Dogs live in an entirely different scent world than we do. They are Profoundly affected by odors we can not detect. In the natural state the dog's sense of smell, olfaction, is used to obtain data important to its survival . The sense of smell, called olfaction involves the detection and perception of chemicals floating in the air. Chemical molecules enter the nose and hair cells are the receptors in the olfactory epithelium that respond to particular chemicals. These cells have small hairs called cilia on one side and an axon on the other side. In humans, there are about 40 million olfactory receptors; in German shepherd dog, there are about 2 billion olfactory receptors. No one knows what actually causes olfactory receptors to react - it could be a chemical molecule's shape or size or electrical charge. The electrical activity produced in these hair cells is transmitted to the olfactory bulb. The information is then passed on to mitral cells in the olfactory bulb. The olfactory tract transmits the signals to the brain to areas such as the olfactory cortex, hippocampus, and hypothalamus. Many of these brain areas are part of the limbic system. The limbic system is involved with emotional behavior and memory.

That's why when you smell something, it often brings back memories associated with the object (Chapman,1960).

#### **1.4.1 Structure and Function of dog olfactory system**

Nasal Plane - The hairless part of the nose, serves as the entry way for scent. It controls the size of the nostril entry passage.

Vomeronasal Organ (VNO) - This organ is not functionally present in the human. (Recent research brings this statement into question and indicates humans may well have a functional vomeronasal organ.) In the dog it is a scent-associated organ located in the roof of the mouth. Nerve fibers connect the vomeronasal gland directly to the olfactory lobes of the dog's brain.

Turbinate - These are bony ridges located inside the nasal passages. They serve several purposes, they slow the movement of the in-coming air, warm it, moisten it, and spread it out over the scent reception area of the nasal chamber. The turbinates are covered with mucous membrane containing the scenting cells.

Sinuses - The sinuses are cavities in the bones near the muzzle of the dog. They are lined with mucous-like cells that may have olfactory capability. The exact purpose and function are not known. It is speculated that their purpose is saving scent for comparison or "memory".

Nerves of Olfaction - These nerves are comparatively large and very numerous. They connect the reception cells/sites with the olfactory lobes of the brain.

Nasal Mucosa - A mucous membrane covers the entire surface of the nasal chambers. The membrane secretes a fluid that serves to moisten incoming air and the surface of the chambers, and acts as a solvent. It also traps particles from the air sample. The mucous is produced by goblet cells.



Olfactory Cells - These cells are critical to the olfactory process. However, it is not known exactly how they interact with the scent particles that reach the nose. There are a number of theories. What is known is that they are present in the nose and linked with the olfactory region of the brain.

Sustentacular Cells - These cells are present where the olfactory cells exist. They appear to have a part in the perception of scent. Some practitioners believe with darker pigmentation do better at scent perception. The dark color of sustentacular cells may support this belief, but for man's purposes most dog's olfactory capacity meets our needs. The sustentacular cells' exact role is not completely understood.

Basal Cells - As suggested by their name the basal cells underlie the sustentacular and olfactory cells. Their function is not clearly understood (Johnston, 1998).

#### **1.4.2 Comparison of Human versus Dog**

The olfactory system is comprised of the nasal chambers, receptor cells, olfactory nerves, and the olfactory lobes of the brain. It is in the olfactory lobes that scent is recognized, processed, and filed to memory. Almost 12% of the dog's brain and 50% of the nasal chambers are devoted to olfaction. In comparison the olfactory lobes of the human brain are much smaller than the dog's. This is also true of the square area of olfactory receptor cells in the human nasal chambers. In the human this area is about the size of a postage stamp compared to about one square yard in a German Shepherd dog. The human receptor site contains approximately 5 million cells where the German Shepherd dog has approximately 220 million cells. Dividing the 5 million into the 220 million would indicate the dog's ability to scent is 44 times greater than the human. However, it may not be that simple, the dog has a larger

processing capacity. When the larger processing capacity is coupled with a data collection system that is 44 times greater the difference may be an exponential of the 44. Since the dog's ability to indicate, if not detect, an odor increases with training this will also widen the gap between man and dog.( Steve, *et al*;1994).

According to ( William ,1985) "almost one-eighth of the dog's brain and over 50% of the internal nose is committed to olfaction, whereas the human olfactory lobes are very much smaller, and the area of olfactory cells is only about one square inch.

Among other findings this indicates why a dog has such a discriminating scent. In that area of his nose it is believed that a man has 5 million olfactory cells compared to a dogs approximately 220 million.

The dog's sensitivity to butyric acid was 100 thousand to 100 million times greater than man's ". The most recent information though indicates that dog's sensitivity as compared to man is between 10 to 100 times greater. However, discrepancies occur based on the material and ability of the dog. Although the dogs ability to appropriately discriminate drugs from other substances has led police officers to utilize dogs for narcotics detection, it is this latter factor that comes into play in the defense of criminals in narcotics prosecutions. Although they have been doubts, dogs have been used successfully with relatively accurate results for long enough time that the courts accept their use ( Findlaw , 2000).



**Fig No (1) Crude heroin**

## Chapter Two

### Materials and methods

This study is designed to investigate the effects of smelling *C. sativa* and heroin on police dogs which are used as narcotic detectors.

#### 2-1- Experimental animals

Twenty-six adult German shepherd dogs, males and females, 2-9 years old, weighing between 25-35kg, working in the Police Dogs Administration were selected. Ten dogs were trained for twelve months as *Cannabis sativa* detectors (group1), six dogs were trained for twelve months as heroin detectors (group2), ten dogs never exposed to drugs were used as a control group (group3).

All dogs were kept under veterinary care in the police dogs hospital and observed by veterinarian daily, weighed weekly and received medicines when necessary. Medicines that affect liver enzymes such as glucocorticoids were avoided.

All dogs had the same vaccination program against the following diseases:-

Parvovirus, Hepatitis, Liptospirosis, Canine distemper and Rabies as shown in table(1).

**TABLE (1) : Vaccination program of experimental dogs**

Age (weeks)	Disease
4 wks	Parvovirus
6 wks	Canine distemper, Hepatitis and Parvovirus.
9 wks	Canine distemper, Hepatitis and Parvovirus
12wks	Distemper, Hepatitis, Parvovirus, Liptospirosis and Rabies

**\*The dose on the twelfth weeks will be repeated annually**

A deworming program was done as shown in table (2)

**TABLE (2): Worming program of experimental dogs**

Age (weeks)	Worm
3 wks	Round and tape worms
6 wks	Round worms
9wks	Tape worms
33 wks	Round and tape worms

\*All deworming agents were given orally as tablets (Pyranterlembonat and Epsiprantel ).

Also a dipping program was carried out against external parasites especially from ticks and mite's every week using Cypermethrin solution in water diluted by the formula (1 ml for 1000 ml Water).

There were records for all animals involving birth date, parents, vaccination, dewormer doses, treatment, estrus time in females, pregnancy, parturition and number of litters.

The dogs were fed local dry dogs food which was a mixture of sorghum flour, rice flour, meat meal (powder), raw animal fat, salt and it was mixed together with 20% of the total weight water. These entities were mixed thoroughly and the resulting mixture was pelleted using the house hold meat mincer, then the pelleted meal is oven dried and stored till consumed .Then the product has been coated with liquidized raw animal fat , egg , vitamins and amino acids as additives .

## **2-2- Housing**

All experiments were carried out in a housing system based on individual feeding and management of dogs. It consists of rooms the dimension of each are 2x1x2.5 meters .The floor was built from concrete and the roof was made of wood to reduce the heat stress. The room was opened from the south and north sides but covered with soils.

## **2-3- The routine training program**

The dogs were taken daily for training program from 7:30Am to 9 Am for 12 months, the training program for group (1) was to smell (sniff) Cannabis sativa without cover for 12 weeks, then with leather or plastic cover for 36 weeks, but before any samples collection they were made to smell it without cover for 15 min daily for a period of 15 days and the same thing was done for the heroin group(group2) .The dogs in group(3) (control group) were taken just for walking (smell nothing).

## **The treatment**

According to the treatment, dogs were divided into the following three groups:

1-Group (1): Ten dogs were used to smell C. sativa 70 gm daily for 48 weeks.

2-Group (2): Six dogs were used to smell heroin 2gm daily for 48 weeks.

3- Group (3): Ten dogs as control group (no treatment).

## **2-4- Samples collection**

Blood samples were collected from the cevalic vein, on three different seasons (summer at May at ambient temperature 40° -45°C, winter at December at ambient temperature 15° -20°C, rainy season at August at ambient temperature 37°- 41°C) .

Blood samples were collected into two types of glass tubes :

- 1) Four ml in Lithium heparin glass tubes for the measurement of liver enzymes (alkaline phosphatase (ALP), glutamic oxalo acetic transamines (GOT) and glutamate pyruvate transamines (GPT)) , Creatinine and urea concentration.
- 2) Two ml in fluoride oxalate glass tubes for plasma glucose concentration.

Blood was centrifuged at 3000 rpm for 10 minutes, plasma was then separated and stored at -20° C till used for analysis.

All Blood samples were collected in the morning after 12 hours fasting and before the daily routine exercises.

## **2-5- Biochemical methods**

### **2-5-1- Determination of plasma alkaline phosphatase (ALP)**

Alkaline phosphatase was determined using test kit catalogue No BEDTT07. According to kinetic test optimized (DGKC) method by the recommendation of Haussament, (1977).

#### **Principles**

Kinetic determination of alkaline phosphatase activity according to the following reaction:-



## Procedure

Wave length 405 nm, temperature 37°C, cuvette 1cm light path, zero adjustment air or distilled water, the working reagent for the macro test was 3.0ml and 1.2 ml for the semi micro-test, the sample was 50µL for the macro test and 20 µL for semi micro-test, they were mixed and waited for 1 min, the extinction increase was measured every minute for 1-3min.

## Calculations

The enzyme activity was calculated using the following formula:-

Alkaline phosphatase (unit/L) = 3300x change in absorbance/minute

## 2-5-2-Determination of plasma glutaminoxalo acetic transaminase (GOT)

Plasma GOT activity was determined by colorimetric method kit No: BEDTT 28 described by Bergmeyer, Bowers *et al*; (1976) and Bergmeyer and Wahlfeild, (1978).

## Principles

The enzyme catalyzes the intraocular transfer of an amino group from aspartate to  $\alpha$  – Oxaloglutarate according to the following reaction:

$\alpha$  – Oxaloglutarate + Aspartate  $\xrightarrow{\text{GOT}}$  L-Glutamate + Oxal acetate

Oxal acetate + NADH  $\xrightarrow{\text{MDH}}$  Malate + NAD<sup>+</sup>

## Procedure

Wave length 340nm Hg , temperature 37°C, cuvette 1cm light path , zero adjustment distilled water ,the working reagent for the macro test was 2.0ml and 1.0ml for the semi micro test ,the sample 200 µL for the macro test and 100 µL for the semi micro test , the extinction increase was measured every minute for 1-3min. Enzyme measured by monitoring



the concentration of oxaloacetate hydrazone formed with 2,4 dinitrophenyl hydrazine. Absorbance of samples and standard were read and the activity was determined from the absorbance table (unit / L).

### **Calculations**

Enzyme activity was calculated using the following formula:

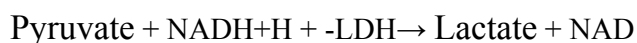
$$\Delta E/\text{min} \times 1750 = \text{U/L}$$

### **2-5-3- Determination of Glutamic pyruvate transaminase (GPT)**

Plasma GPT activity was determined by using test kits catalogue NO.BEDTT 26 according to the method described by Bergmeyer and Walefeld,(1978) and Bergmeyer and Horder ,(1980).

### **Principles**

GPT was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4 dinitrophenyl/hydrazine group from L-alanine to  $\alpha$ -oxalo glutarate according to the following reaction :



### **Procedure**

Wave length 340nm Hg , temperature 37°C, cuvette 1cm light path , zero adjustment distilled water ,the working reagent for the macro test was 2.0ml and 1.0ml for the semi micro test ,the sample 200  $\mu$ L for the macro test and 100  $\mu$ L for the semi micro test , the extinction increase was measured every minute for 1-3min. The rate of NADH consumption is determined photometrically and is directly proportional to the GPT (ALT) activity in the sample.

### **Calculations**

Absorbance of sample and standard were read against the reagent blank.

The enzyme activity was calculated using the following formula:

$$\Delta E/\text{min} \times 1750 = \text{U/L}.$$

#### **2-5-4-Determination of glucose**

Glucose level was measured by enzymatic colorimetric test kit Plasma (GOD-PAP) described by Tindler, (1969) Dingee, (1975) and Lott, (1975).

##### **Principles**

Glucose is oxidized by glucose – oxidase to gluconate and hydrogen peroxide according to the following reaction:



##### **Procedure**

Absorbencies and standard were read against reagent blank at:  
Wave length 505nm, temperature 37°C, cuvette 1cm light path, zero adjustment reagent blank, incubation 10 min.

##### **Calculation**

The glucose concentration was calculated using the following equation:

$$\text{Glucose (mg/dl)} = \frac{\text{Optical density sample}}{\text{Optical density standard}} \times \text{standard concentration}$$

Standard concentration = 100 mg/dl

#### **2-5-5- Determination of urea**

Plasma urea level was measured by the enzymic colorimetric test kit (Berthelot).

Urase Berthelot reaction method described by Berthelot, (1859) and Bushman, (1960).

##### **Principle**

Urea is hydrolyzed into ammonia and CO<sub>2</sub>. Ammonia reacts with salicylate and hypochlorite to form a green indo phenol . The colour intensity is proportional to the concentration of urea according to the following reaction:-



The ammonium ions formed in the reaction react with salicylate hypochlorite to form 2,2-dicarboxylindophenol, which is green in colour.

### **Procedure**

Absorbencies and standard were incubated for 5min at 37°C, the extinction was measured at 580 nm against blank.

### **calculation**

Urea concentration was calculated using the following equation:

$$\text{Urea (mg/dl)} = \frac{\text{O.D of samples}}{\text{O.D of standard}} \times \text{standard concentration.}$$

### **2-5-6- Determination of Creatinine**

Creatinine was measured using test kit catalogue No BSDTT13 according to the method described by Hare ,(1950) , Kostir and Sonka (1952) .

### **Principles**

Creatinine in a basic picrate solution forms a colored complex. The O.D extinction at predetermination times during conversion is proportional to the concentration of creatinine in the sample.

### **Procedure**

Serum 100 µL , Standard 100 µL, Reagent mixture 1.0ml .The sample and standard were read(E1) after 30 sec at 492 nm, then they were read again (E2) after 1 min .Having calculated the  $\Delta E = (E2 - E1)$  the sample

creatinine concentration will be obtained using the following formula :  
creatinine (mg/dl) =  $\frac{(E2 - E1)}{\text{standard}}$  X Conc. .Standard mg/dl.

(E2-E1) standard

---





**Fig No (2) German Shepherd during searching for C-sativa .**



**Fig No (3) German Shepherd found the C-sativa and holding it .**

## **CHAPTER THREE**

### **RESULTS**

#### **3.1 The effects of narcotic detection by smelling on some biochemical parameters in German shepherd dogs.**

##### **3.1.1 Liver enzymes**

The effects of narcotic detection by smelling on liver enzymes is presented in table (3).

In all German shepherd detectors the mean values of plasma alkaline phosphatase and glutamic pyruvate transaminase showed similar level compared to the control group, whereas the mean values of glutamic oxaloacetic transaminase (GOT) was slightly higher in C.sativa group compared to the control but not significant.

##### **3.2.1. Plasma urea and creatinine**

The effects of narcotic detection by smelling on the level of urea and creatinine is presented in table (3).

In all German shepherd narcotic detectors the mean values of plasma urea showed slight increase compared to the control, but the

difference was not significant. The same effect was also observed in the plasma creatinine which was slightly elevated in the heroin detectors.

**Table (3) : The effect of narcotic detection by smelling on different biochemical parameters in German shepherd dogs ( mean  $\pm$  SE) .**

<b>Parameters Treatment</b>	<b>GOT u/l</b>	<b>GPT u/l</b>	<b>ALP u/l</b>	<b>Urea mg /dl</b>	<b>Creatinine mg /dl</b>	<b>Glucose mg /dl</b>
<b>Cannabis</b>	26.776 <sup>a</sup> $\pm$ 2.292	31.33 <sup>a</sup> $\pm$ 2.187	85.170 <sup>a</sup> $\pm$ 9.107	25.920 <sup>a</sup> $\pm$ 1.966	1.194 <sup>a</sup> $\pm$ 0.060	80.753 <sup>a</sup> $\pm$ 3.060
<b>Heroin</b>	23.33 <sup>a</sup> $\pm$ 3.179	32.833 <sup>a</sup> $\pm$ 3.033	87.167 <sup>a</sup> $\pm$ 12.631	25.555 <sup>a</sup> $\pm$ 2.727	1.212 <sup>a</sup> $\pm$ 0.084	83.362 <sup>a</sup> $\pm$ 2.244
<b>Control</b>	23.72 <sup>a</sup> $\pm$ 2.112	32.303 <sup>a</sup> $\pm$ 2.015	87.470 <sup>a</sup> $\pm$ 8.392	24.813 <sup>a</sup> $\pm$ 1.812	1.164 <sup>a</sup> $\pm$ 0.055	83.395 <sup>a</sup> $\pm$ 2.820

\*Means within the same column having different superscript letters are significantly different at (P< 0.05)



### 3.1.3 Plasma glucose

In German shepherd C.sativa detectors the mean values of plasma glucose showed not significant slight decrease compared to the control. But the level of glucose was not affected in heroin detectors as showed in table (3).

## 3.2 The effects of sex, age and season on plasma parameters of narcotic detectors

### 3.2.1 Plasma glucose in narcotic detectors dogs

The effects of sex, age and season on plasma glucose are presented in table (4,5 and 6 ).

Plasma glucose levels were found to be lower in females' detectors dogs compared to males. This sex difference was not observed in the control groups. Though all differences were not significant.

**Table (4): Effects of treatment and sex on plasma glucose (mg/dl) in German shepherd dogs (Mean + SE).**

Sex	Male	Female
Treatment		
Cannabis sativa	81.183 <sup>a</sup> $\pm$ 1.741	80.33 <sup>a</sup> $\pm$ 0.972
Heroin	84.67 <sup>a</sup> $\pm$ 1.65	82.10 <sup>a</sup> $\pm$ 1.400
Control	83.23 <sup>a</sup> $\pm$ 0.833	83.41 <sup>a</sup> $\pm$ 1.741

Means having different superscript letters in the same raw are significantly different ( $P < 0.05$ )

**Table (5) : Effects of treatment and age on plasma glucose (mg/dl) in German shepherd dogs (Mean  $\pm$  SE) .**

Age	2- 4 years	5-7 years	8- 9 years
Treatment			
Cannabis sativa	79.67 <sup>a</sup> $\pm$ 1.038	81.00 <sup>a</sup> $\pm$ 1.741	80.70 <sup>a</sup> $\pm$ 1.270
Heroin	82.90 <sup>a</sup> $\pm$ 1.400	84.73 <sup>a</sup> $\pm$ 1.121	83 <sup>a</sup> .00 $\pm$ 1.111
Control	83.93 <sup>a</sup> $\pm$ 1.121	82.67 <sup>a</sup> $\pm$ 1.212	83.25 <sup>a</sup> $\pm$ 1.232

Means having different superscript letters in the same raw are significantly different (P < 0.05).

No significant differences were observed in the plasma glucose levels of the three age groups studied in this work. Only slightly higher levels were noticed in the middle age group compared to the older and younger ones.

**Table (6): Effect of treatment and season on plasma glucose (mg/d) in German shepherd dog (Mean + SE).**

Age	Autumn	Summer	Winter
Treatment			
Cannabis sativa	81.20 <sup>a</sup> $\pm$ 1.400	80.67 <sup>a</sup> $\pm$ 1.400	80.33 <sup>a</sup> $\pm$ 1.4
Heroin	82.25 <sup>a</sup> $\pm$ 2.619	83.25 <sup>a</sup> $\pm$ 2.619	84.60 <sup>a</sup> $\pm$ 2.619
Control	83.00 <sup>a</sup> $\pm$ 1.232	83.18 <sup>a</sup> $\pm$ 1.232	84.00 <sup>a</sup> $\pm$ 1.232

Means having different superscript letters in the same raw are significantly different (P < 0.05)

When plasma glucose concentration, was measured in the narcotics detectors in the three seasons, no significant differences were observed. The same levels of plasma glucose kept constants during summer, winter and autumn.

### 3.2.2 Plasma creatinine

The level of plasma creatinine in both males and females, old and young dogs was in the normal range as showed in tables (7, 8). Whereas, the level of plasma creatinine decreased significantly in winter compared to summer and autumn as showed in table ( 9 ) in both detectors and non detectors German shepherd dogs.

**Table (7): Effects of treatment and sex on plasma creatinine (mg/dl) in German shepherd dogs (Mean + SE) .**

<b>sex Treatment</b>	<b>Male</b>	<b>Female</b>
Cannabis sativa	1.283 <sup>a</sup> ± 0.741	1.102 <sup>a</sup> ± 0.972
Heroin	1.30 <sup>a</sup> ± 0.672	1.13 <sup>a</sup> ± 0.410
Control	1.12 <sup>a</sup> ± 0.833	1.20 <sup>a</sup> ± 0.741

Means having different superscript letters in the same raw are significantly different ( $P < 0.05$ )

Males showed non significantly higher plasma creatinine levels compared to females in the detectors groups, but this difference was not observed in the control males and females. Also higher levels were observed in detectors groups , compared to plasma creatinine level in younger detectors .

In the detectors groups the plasma creatinine levels were higher during autumn and lower during winter. Whereas in the control groups the highest level was observed during summer season.

**Table (8): Effects of treatment and age on plasma creatinine (mg/dl) in German shepherd dogs (Mean + SE).**

Age	2-4 years	5-7 years	8-9 years
Treatment			
Cannabis sativa	1.178 <sup>a</sup> ± 0.38	1.167 <sup>a</sup> ± 0.741	1.233 <sup>a</sup> ± 0.645
Heroin	1.222 <sup>a</sup> ± 0.4	1.124 <sup>a</sup> ± 0.4	1.300 <sup>a</sup> ± 0.38
Control	1.167 <sup>a</sup> ± 0.121	1.167 <sup>a</sup> ± 0.4	1.133 <sup>a</sup> ± 0.317

Means having different superscript letters in the same raw are significantly different (P < 0.05)

**Table (9): Effects of treatment and season on plasma creatinine (mg/dl) in German shepherd dogs (Mean + SE)**

Age	Autumn	Summer	Winter
Treatment			
Cannabis sativa	1.433 <sup>a</sup> ± 0.400	1.163 <sup>a</sup> ± 0.3	0.978 <sup>b</sup> ± 0.356
Heroin	1.40 <sup>a</sup> ± 0.287	1.25 <sup>a</sup> ± 0.1	1.00 <sup>b</sup> ± 0.346
Control	1.258 <sup>a</sup> ± 0.239	1.30 <sup>a</sup> ± 0.202	0.930 <sup>b</sup> ± 0.267

Means having different superscript letters in the same raw are significantly different (P < 0.05)

### 3.2.3. Plasma urea

The level of plasma urea in both males and females ,old and young dogs increased significantly in summer and autumn compared to winter as shown in tables (10 ,11 and12 ) in both treated and the control German shepherd dogs .

**Table (10): Effects of treatment and sex on plasma urea (mg/dl) in German shepherd dogs (Mean + SE).**

Age	Male	Female
Treatment		
Cannabis sativa	25.33 <sup>a</sup> ± 1.741	26.66 <sup>a</sup> ± 0.972
Heroin	25.97 <sup>a</sup> ± 0.741	24.92 <sup>a</sup> ± 1.400
Control	25.00 <sup>a</sup> ±0.833	24.626 <sup>a</sup> ± 1.741

Means having different superscript letters in the same raw are significantly different (P< 0.05)

**Table (11): Effects of treatment and age on plasma urea (mg/dl) in German shepherd dogs (Mean + SE).**

Age	2-4 years	5-7 years	8-9 years
Treatment			
Cannabis sativa	25.72 <sup>a</sup> ± 1.038	25.83 <sup>a</sup> ± 1.741	26.20 <sup>a</sup> ± 1.038
Heroin	24.22 <sup>a</sup> ± 1.400	27.70 <sup>a</sup> ± 1.121	24.67 <sup>a</sup> ± 1.038
Control	24.60 <sup>a</sup> ± 0.121	25.08 <sup>a</sup> ± 1.400	24.71 <sup>a</sup> ± 1.232

Means having different superscript letters in the same raw are significantly different (P < 0.05)

**Table (12): Effects of treatment and season on plasma urea (mg/dl) in German shepherd dogs (Mean + SE).**

Age	Autumn	Summer	Winter
Treatment			
Cannabis sativa	29.98 <sup>a</sup> ± 1.400	28.58 <sup>a</sup> ± 1.400	19.20 <sup>b</sup> ± 1.4
Heroin	30.25 <sup>a</sup> ± 2.619	28.20 <sup>a</sup> ± 2.619	18.201 <sup>b</sup> ± 2.619
Control	28.03 <sup>a</sup> ± 1.232	26.83 <sup>a</sup> ± 1.232	19.58 <sup>b</sup> ± 1.232

Means having different superscript letters in the same row are significantly different (P< 0.05)

### 3.2.4 Plasma alkaline phosphatase (ALP)

The level of plasma alkaline phosphates in both males and females, old and young dogs was in the same level compared to control, but increased significantly in summer and autumn compared to the winter as shown in tables (13, 14 and 15) in both treated and control German shepherd dogs.

**Table (13): Effects of treatment and sex on plasma alkaline phosphatase (u/l) in German shepherd dogs (Mean + SE).**

Age	Male	Female
Treatment		
Cannabis sativa	85.83 <sup>a</sup> ± 1.741	84.51 <sup>a</sup> ± .972

Heroin	87.15 <sup>a</sup> ± 0.741	87.18 <sup>a</sup> ± 1.400
Control	87.68 <sup>a</sup> ± 0.833	87.23. <sup>a</sup> ± 1.741

Means having different superscript letters in the same raw are significantly different (P < 0.05)

**Table (14): Effects of treatment and age on plasma alkaline phosphatase (u/l) in German shepherd dogs (Mean + SE).**

Age	2-4 years	5-7 years	8-9 years
Treatment			
Cannabis sativa	84.26 <sup>a</sup> ± 1.038	85.06 <sup>a</sup> ± 1.741	86.17 <sup>a</sup> ± 1.038
Heroin	87.18 <sup>a</sup> ± 1.40	87.17 <sup>a</sup> ± 1.121	87.33 <sup>a</sup> ± 1.321
Control	86.87 <sup>a</sup> ± 1.121	86.89 <sup>a</sup> ± 1.40	88.542 <sup>a</sup> ± 1.232

Means having different superscript letters in the same raw are significantly different (P < 0.05)

**Table (15): Effects of treatment and season on plasma alkaline phosphatase (u/l) in German shepherd dogs (Mean + SE).**

Age	Autumn	Summer	Winter
Treatment			
Cannabis sativa	86.05 <sup>a</sup> ± 1.4	88.18 <sup>a</sup> ± 1.4	81.28. <sup>b</sup> ± 1.4
Heroin	88.00 <sup>a</sup> ± 2.619	92.25 <sup>a</sup> ± 2.619	81.25 <sup>b</sup> ± 2.618
Control	88.83 <sup>a</sup> ± 1.232	90.75 <sup>a</sup> ± 1.232	82.83 <sup>b</sup> . ± 1.232

Means having different superscript letters in the same raw are significantly different (P< 0.05).

The highest level of alkaline phosphatase was observed during summer for all groups. Whereas, the lowest levels were reported during the winter season.

### 3.2.5 Plasma glutamate oxaloacetate transaminase (GOT)

The level of plasma glutamate oxalo acetate in both males and females, old and young dogs was similar to control, but increased significantly in summer and autumn compared to the winter as shown in table (16,17and18 ) in both treated and control German shepherd dogs.

**Table (16): Effects of treatment and sex on plasma glutamate oxaloacetate transaminase (u/l) in German shepherd dogs (Means+SE) .**

<b>sex Treatment</b>	<b>Male</b>	<b>Female</b>
Cannabis sativa	27.20 <sup>a</sup> ± 1.741	26.29 <sup>a</sup> ± 0.972
Heroin	22.06 <sup>a</sup> ± 0.741	24.22 <sup>a</sup> ± 1.4
Control	23.10 <sup>a</sup> ± 0.833	24.41 <sup>a</sup> ± 1.741

Means having different superscript letters in the same raw are significantly different (P < 0.05).

**Table (17): Effects of treatment and age on plasma glutamate oxaloacetate transaminase (u/l) in German shepherd dogs (Mean± SE).**

<b>Age Treatment</b>	<b>2-4 years</b>	<b>5-7 years</b>	<b>8-9 years</b>
Cannabis sativa	27.91 <sup>a</sup> ± 1.038	26.83 <sup>a</sup> ± 1.741	25.57 <sup>a</sup> ± 1.038
Heroin	23.22 <sup>a</sup> ± 1.4	23.07 <sup>a</sup> ± 1.121	23.02 <sup>a</sup> ±1.212



Control	23.80 <sup>a</sup> ± 1.121	22.72 <sup>a</sup> ± 1.4	24.74 <sup>a</sup> ± 1.232
---------	----------------------------	--------------------------	----------------------------

Means having different superscript letters in the same raw are significantly different (P <0.05).

**Table (18): Effects of treatment and season on plasma glutamate oxaloacetate transaminase (u/l) in German shepherd dogs (Mean±SE).**

season	Autumn	Summer	Winter
Treatment			
Cannabis sativa	29.11 <sup>a</sup> ± 1.4	29.89 <sup>a</sup> ± 1.4	21.33 <sup>b</sup> ± 1.4
Heroin	25.15 <sup>a</sup> ± 2.619	25.00 <sup>a</sup> ± 2.219	19.25 <sup>b</sup> ± 2.619
Control	25.25 <sup>a</sup> ± 1.232	26.58 <sup>a</sup> ± 1.232	19.33 <sup>b</sup> ± 1.232

Means having different superscript letters in the same raw are significantly different (P < 0.05).

### 3.2.6 Plasma glutamate pyruvate transaminase

The level of plasma glutamate pyruvate transaminase in both males and females ,old and young dogs was similar to control , but increased significantly in summer and autumn compared to the level in winter as showed in table (19,20and 21 ) in both treated and untreated German shepherd dogs.

**Table (19): Effects of treatment and sex on plasma glutamate pyruvate transaminase (u/l) in German shepherd dogs(Mean + SE).**

sex	Male	Female
Treatment		

Cannabis sativa	32.24 <sup>a</sup> ± 1.741	30.42 <sup>a</sup> ± 0.972
Heroin	32.446 <sup>a</sup> ± 0.741	33.22 <sup>a</sup> ± 1.4
Control	32.00 <sup>a</sup> ± 0.833	32.606 <sup>a</sup> ± 1.741

Means having different superscript letters in the same row are significantly different (P < 0.05)

**Table (20): Effects of treatment and age on plasma glutamate pyruvate transaminase (u/l) in German shepherd dogs (Mean + SE).**

<b>Age Treatment</b>	<b>2-4 years</b>	<b>5-7 years</b>	<b>8-9 years</b>
Cannabis sativa	32.00 <sup>a</sup> ± 1.038	31.50 <sup>a</sup> ± 1.741	30.50 <sup>a</sup> ± 1.038
Heroin	32.22 <sup>a</sup> ± 1.212	32.90 <sup>a</sup> ± 1.121	33.51 <sup>a</sup> ± 1.23
Control	33.53 <sup>a</sup> ± 1.121	31.97 <sup>a</sup> ± 1.40	31.60 <sup>a</sup> ± 1.232

Means having different superscript letters in the same row are significantly different (P < 0.05)

**Table (21): Effects of treatment and season on plasma glutamate pyruvate transaminase (u/l) in German shepherd dogs (Mean+ SE).**

<b>season Treatment</b>	<b>Autumn</b>	<b>Summer</b>	<b>Winter</b>
Cannabis sativa	33.44 <sup>a</sup> ± 1.400	32.00 <sup>a</sup> ± 1.400	28.56 <sup>b</sup> . ± 1.400
Heroin	35.25 <sup>a</sup> ± 2.619	34.75 <sup>a</sup> . ± 2.619	28.50 <sup>b</sup> ± 2.619
Control	35.75 <sup>a</sup> ± 1.232	34.33. <sup>a</sup> ± 1.232	26.83 <sup>b</sup> ± 1.232

Means having different superscript letters in the same row are significantly different ( $P < 0.05$ )

## **Chapter four**

### **Discussion**

#### **4.1 The effects of narcotic detection by smelling on some biochemical parameters in German shepherd dogs**

##### **4.1.1 Liver enzymes**

The levels of liver enzymes are the most important factors to investigate liver damage and injuries .Hoffman, *etal*; (1977) discovered that plasma alkaline phosphatase activity has an important role for characterizing bone and hepatic disorders , when obstruction of the duct system occurs at any level and hepatic fibrosis was increased , hepatic alkaline phosphatase activity in serum increased ,but more common occurrence is its increase association with hepatic lipidosis and severe starvation .Hussein, (2003) found that rats injected with C.sativa and addicts men showed slight non significant increase in plasma alkaline phosphatase .In the present study in all German shepherd C-sativa detectors the mean values of plasma alkaline phosphatase showed similar

Level compared to the control group (Table 3) and this result may be influenced by the number of dogs in this study. The level of (SGOT) was slightly higher compared to the control whereas, Hussein, (2003) found that rats injected with C-sativa extract showed significantly higher level of (SGOT) and the two studies are in agreement if we take in consideration that she injected the animals and in this study they were treated by smelling . Glutamic pyruvate transaminase (SGPT) showed similar levels compared to the control group in the present study(Table 3), Cornelius and Kaneko , (1986) reported that the level of( SGPT) in the blood is used as an indicator for hepatocytes integrity ,disrupt hepatic parenchyma cells, with necrosis or altered membranes permeability, will lead to the leakage of the enzyme to

the blood stream , in this study little changes were observed on the levels of the enzyme , this is may be due to the small number of the experimental groups , and the route of administration.

Pearce, (1980) reported that heroin abuse may have resulted in impaired liver function. Also heroin addicts showed high level of GOT, GPT and alkaline phosphatase (Gruskin,1999 ). In other work when rats injected with heroin 20mg weekly for three weeks, serum alkaline phosphates (ALP), glutamic oxalo acetic transaminase (GOT), glutamic pyruvate transaminase (GPT) increased significantly, then returned to the normal level after 14 days (Baltimore ,1993 ). In this study heroin detectors showed similar level for all enzymes compared to control (Table3) this result could be found to be different in longer time and more numbers of animals.

#### **4.1.2 Plasma urea and creatinine**

Heroin induced is a potent vasoconstrictor, causes rhabdomyolysis by limiting the blood supply to the muscle, rhabdomyolysis elevates urea and creatinine into blood stream, which lead to high urea and creatinine levels in urine (Darke, *et al.*, 1996).

When hashish or one of its active constituents are administrated to animals , marked accumulation of the injected material take place in the liver (Dingell, *etal*;1974 ,Jakubovic and Mcgeer ,1972) . Exposure of rabbits to hashish smoke every other day for a period of one month resulted in marked increase in blood ammonia ,and increased blood urea concentration in these animals . Possibility for the raised blood levels , would be the assumption that there is an increased rate of amino acids breakdown in these animals leading to the formation of a large amount of ammonia , to stimulate the activity of enzymes involved in urea biosynthesis and

increased urea level and rabbdomylosis increase plasma creatinine, (Ghoneim,*etal*;1980) .Tetrahydro canabinol produced no changes on serum urea nitrogen in guinea pig treated with delta 9- THC at the rate of 3 mg / kg Bwt daily for six month (Huy,*et al* ; 1975). This is in agreement with results obtained in this study, in all German shepherd C-sativa detectors the mean values of plasma urea and creatinine showed an increase compared to the control level, but it was not significant and the same effect was also observed in the heroin detectors German shepherd dogs. These findings suggest that, olfaction of narcotics has very little effect on the kidney function.

#### **4.1.3 Plasma glucose**

Blood glucose is derived from the diet, gluconeogenesis, glycogenolysis and insulin play a central role in the regulation of blood glucose level and glucagon oppose the action of it (Murray, *etal*; 2000). Permutt, *etal*; (1976) studied the effect of marihuana on carbohydrate metabolism in fed and fasting states in chronic marihuana users. They did not record hypoglycemia in 7 patients who were given marihuana after fasting for 24-72 hours .They also found no significant differences in carbohydrate tolerance and no hypoglycemia during an oral glucose tolerance test in 10 patients who smoked marihuana on alternate days .In this study all German shepherd C.sativa detectors showed mean values of plasma glucose of slight decrease compared to the control group (table 3),this non significant effect could be due to the smelling of the C.sativa and this result may be changed if we use more number of experimental animals and more longer time .

Heroin addiction seems to produce contemporaneously a state of hyperinsulinaemia. It is concluded that chronic heroin addiction may produce a change in the rate of hepatic extraction of insulin. In heroin addicts the glycemic response to the glucose load shows a delayed peak time. The insulin curves show increased insulin peaks, delayed peak time and prolonged hyper insulinemia and significant decrease of plasma glucose (Foltin,*etal*;1986).In this study the level of glucose was not affected in heroin detectors as showed in table (3) and this result indicates that smelling heroin dose not affect insulin production in the  $\beta$ -cells.

## **4.2 The effects of sex, age and season on plasma parameters of narcotic detectors**

### **4.2.1 Plasma creatinine**

In the present study the level of plasma creatinine decreased significantly (  $p < 0.05$  ) in winter compared to summer and autumn as showed in table ( 9 ) in both detectors and non detectors German shepherd dogs .This result is in good agreement with (Clowes,*etal*; 1974) who observed that heat stress lead to rhabdomyolosis and then high plasma creatinine .Also Knochel ,(1987 ) found that heat stress can lead to serious damage to the kidneys of German shepherd dogs .

No clear effect was observed in the present work due to narcotics smelling and sex, only higher levels of plasma creatinine was observed in older age animals of the narcotic detectors compared to younger groups. This effect was not detected in the control groups.

#### **4.2.2 Plasma urea**

In this study plasma urea level in both males and females ,old and young dogs increased significantly( $p < 0.05$  ) in summer and autumn compared to the winter as shown in table (12 ) in both treated and un treated German shepherd dogs , similar result was detected by (Clowes, *etal*;1974) who found that heat stress as the high temperature in summer may lead to rhabdomyolysis and then high level of plasma urea in dogs . Urea levels in the present work were not affected by age or sex difference.

#### **4.2.3 Plasma alkaline phosphatase ( ALP )**

In the present study the level of plasma alkaline phosphates in both males and females ,old and young dogs was in the same level, but increased significantly in summer and autumn compared to winter as showed in table (13 ,14 , 15 ) in both treated and un treated German shepherd dogs . This result can be explained as effect of high temperature during the two hot seasons (summer and autumn) in Sudan. Then season affected liver of all animals in this work. . This result is in good agreement with the results obtained by(Clowes,*etal*;1974) who found that hyperthermia lead to hepatic injury, increases plasma alkaline phosphatase and Sawka, (1985) also observed that in dogs ,hyper thermia may lead to hepatocellular damage that elevates serum enzymes such as alkaline phosphatase .

#### **4.2.4 Plasma glutamic oxalo acetic transaminase ( GOT )**

Clowes, *et al* ; (1974) and Sawka ,(2001 ) mentioned that heat stress (high temperature ) causes liver damage and elevates liver enzymes such as



glutamic oxalo acetic transaminase ( GOT ) . In the present study the level of this enzyme increased significantly ( $p < 0.05$ ) in summer and autumn compared to winter as showed in tables (16, 17 and 18).

#### **4.2.3 Plasma glutamic pyruvic transaminase ( GPT )**

The level of plasma glutamic pyruvic transaminase in both males and females ,old and young dogs was within the same level of the control group, but increased significantly in summer and autumn compared to the winter as shown in table (19,20 and21 ) in both treated and un treated German shepherd dogs . This result is in agreement with Clowes, *etal*; (1974) and Sawka , (2001 ) who mentioned that heat stress (high temperature ) causes liver damage and elevates liver enzymes such as Plasma glutamic pyruvic transaminase ( GPT ) .

Previous studies showed that narcotics consumption with heat stress significantly affects liver and renal function (Hoffman,*etal*;1977), Cornelius and Kaneko, (1986) Ghoneim, *etal*; (1980) .In the present work a clear effect of elevation of environmental temperature, during summer and autumn, was observed in shepherd dogs. This was presented as significant ( $p < 0.05$ ) increase of important plasma measures for liver and renal function such as creatinine, urea and the liver enzymes. Since these parameters are slightly elevated in the narcotic detectors compared to the control in present study, this implies that, dogs trained in hot days may suffer renal and liver problems. Also measuring these values in large numbers of animals and for longer periods will be of great value to evaluate the level of the risk.

## CONCLUSION

The present study was carried out to investigate the effects of smelling narcotics by German shepherd dogs for detection training on certain biochemical parameters in Sudan Police Dogs Administration in three different seasons (summer, autumn and winter).

The mean values of plasma liver enzymes (ALP) and (GPT) were not affected by smelling narcotics, whereas the mean values of (GOT) were slightly higher compared to the control. Heroin detectors showed similar levels for all enzymes compared to control. For the kidney function the mean values of plasma urea and creatinine showed slight increase compared to the control in *C.sativa* detectors, but not significant, the same effect was also observed in the heroin detectors. The mean values of plasma glucose showed slight decrease compared to the control, but the level of glucose was not effected in heroin detectors.

The seasonal variations were very clear in the present study, plasma creatinine, liver enzymes (ALP, GPT and GOT) and plasma urea were significantly high ( $p < 0.05$ ) in summer and autumn compared to winter in all groups.

### Recommendations:-

- 1- The effect of narcotics detection in these useful animals should be intensively studied, literature before this work, is not available.
- 2- The effects of high temperature in Sudan on German shepherd dogs should be considered during the training.
- 3- The relationship and effects of narcotic detection and high temperature, also the best times and seasons to train narcotics detectors in Sudan, should be carefully determined.

## REFERENCES

Annon, Z. (1970). Clin .Chem.a .Clin .Biochem 8; 658.

Baltimore, M.D. (1993). Heroin toxicity. Bri. J of Anes.Vol 85. No 1. pp 136- 142.

Bergmeyer, Bower and Cols. (1976). Determination of AST. Clin. Chem.Acta 70, F 19-42.

Bergmeyer and Wahlefeld. (1978). Determination of ALT .Clin. Chem. 24, 58-73

Bergmeyer and Holder. (1980). GPT determination. Clin. Chem .Acta 105-147.

Berthelot, M.P. (1859). Urase Berthelot reaction method. Repert Chem. Appl ,2884.

Blevins, R.D. (1976). Heroin effect on macromolecular synthesis in human and other mammalian cells. Arch.Tox: 35 (2). 127-135.

Booth, M. (1996). Opium. A History. London; Simon .Schuster, Ltd.

Bushman, C. J, Balleter WG and Tidwell . B.W. (1960).Determination of urea. Anal .Chem. 33, 592.

Carlton, E .T. (1983).Cannabis; the plant; its drugs and their effects. Aviation, space and environmental medicine. 363-368.

Chapman, S.G. (1960). Dogs in Police work. Public Administration Services.

Clowes, G.H.; O'Donnell T.F. (1974). Heat stroke. Engl .J. Med.291, 564–567.

Cohen, G .M and Peterson, G .J. (1971). Mannering; Interaction of delta -9-THC with the hepatic microsomal drug metabolizing system. Life Sci 10, 1207.

Cohen, S. (1982). Cannabis; effects on adolescent motivation. From Marijuana and Youth. Clinical Observation on Motivation and Learning. Rockville. Maryland. National Institute on Drug Abuse. 2-11.

Cone, E.J; Johnson R.E; Paul, B.D. (1988). Marijuana-laced brownies; behavioral effects; physiologic effects, and urinalysis in humans following ingestion. J Anal Toxicol 12 (4); 169-175.

Cornelius, C.E and Kaneko, J.J. (1986) .Clinical biochemistry of domestic animals .Academic Press .New York and London.

Darke, S; Zador, D.(1996). Fatal heroin 'overdose; a review. Addiction 91(12): 1765-72.

David, F. (1987). The American Disease; Origins of Narcotic Control. New York; Oxford University Press.

Dingell, I.V; Miller, K .W; Health, E .C and Klausner, H.A. (1974). Biochem .Pharmacol. 22; 949.

Dingeon, B. (1975). Bio .Clin. 33, 3 .

Ellen, T.Y and Jonathan,E.G . (1973). Cannabis Rx ;the Cannabis Institute of American Inc.Vol .1;No.1;pp.1-4.

Ellenhorn, S; Hall, W; Weatherburn, D. (1999) Fluctuations in heroin purity and the incidence of fatal heroin overdose. Drug Alcohol Depend .54(2): 155-61.

Findlaw, S and Terry, V. (2000). Ohio; Certiorari to the Supreme Court of Ohio.

Foltin, R.W; Brady, J.V., and Fischman, M.W. (1986). Behavioral analysis of heroin effects on food intake in humans. Pharmacol Biochem. Behav. 25:577.

George, R.N and John, I.T. (1973). The forensic Identification of marijuana; Some question and answers; J. of Pol. Sci and Admin .1: 102-111.

Ghoneim, M.T; Mikhail, M.M; Mahfouz, M .and Maker, A.B. (1980).Effects of Hashish smoke on some blood and serum parameters in rabbits. Pharmizie 35 (4): 226-8.

Gruskin, J.M. (1999); heroin hepatotoxicity Bri J of Anaeth.85; (1) 136-142.

Hardin, J and Arena, J. (1974). Human poisoning from native and cultivated plants 2nd. Ed. Duke University Press; Durham; (USA).

Hare, R. S. (1950) .Endogenous creatinine in serum and urine Pro. Soc. Exp .Biol .Med 74, 148.

Haussament, T .U. (1977) cols .Clin .Chem .Acta, 35; 271-273.

Hoffman, W.E; Renegar ,W.E and Doner,J.L.(1977).Clinical important of alkaline phosphatase. Vet.Res.38,1637-1639.

Hollister, L.E. (1988). Cannabis. Acta Psychiatric Scand Suppl 345: 108-118.

Hussein, A .M. (2003).Biochemical and immunological effects of Cannabis Sativa in rats and men. Thesis U of K .p 64.

Huy ,N.D ; Gailis, L ;Cote, G .and Roy P.E. (1975) .Effects of chronic administration of delta -9-tetrahydrocannabinol (Delta-9-THC) In Guinea pigs . Int .J.Clin.Pharmacol .Biopharm12(1-2):284-289.

Jaffe, J.H. ; Goodman, L.S and Gilman, A. (1986). Drug Abuse ."Bases Pharmacological of Cannabis (1); 17.

Jakubovic , A and Mcgeer .Canada ,P .L. (1972). J. Biochem .50, 654.

Johansson, E; Agurell, S; Hollister, L.E; Haldin ,M.M.(1988). Prolonged apparent half-life of delta-9- tetrahydrocannabinoid in plasma of chronic marijuana users. 40 (5): 374-375).

Johnston, M.T. (1998). Dog olfaction .Institute for Biological Detection System.

Kabelik, K.J; Krejei, Z and Santavy, F. (1960). "Cannabis an amedicament" Bulletin on narcotics, X11, No.3, 5-23.

Kephalis, T .A; Kiburis, J; Michael, C .M; Miras ,C .J and Papadakis,D.P. (1976) . Some aspects of Cannabis smoke chemistry. In Marijuana Chemistry; Biochemistry and cellular effects; G.G Nahas (eds), pp.39-49.Springer-verlag; New York.

Kostir, J.V and Sonka,J .A.(1952) .New method Biochim Biophys .Acta (Amst) 8,86.

Knochel, J.P; Reed, G; Kleeman, C.R; Maxwell, M.H; Nari, R.G (1987). Disorders of heat regulation. In editors; Clinical disorders of fluid and electrolyte metabolism. New York; McGraw-Hill.

Lemberger, L; Memahon, Rand Archer; R. (1976). The role of on version on mechanism of action of cannabinoids.In Pharmacol of Marihuana, M.C.Brude and S.Szara (eds) pp.125-135.Raven Press ; New York.

Lott , J .A .(1975). Determination of glucose .Clin .Chem .21, 1754.

Madelaine, O.M. (1985). Health consequence of acute and chronic marijuana use. Prog. Neutro . Psycholopharmacol Biol .Psychiat 209-238 .  
Mahfouz , M ; Maker ,A .B ;Ghoneim, M .T .and Makhail, M. M .(1975). Effects of Hashish on brain gamma aminobutyric acid system ,blood fibrinolytic activity and some serum enzymes in rats .Pharmazie ,30 (12) 772-4.

Malik, S.A., Khan, C., Jabbar, A., Iqbal, A ; (1992) . Heroin addiction and sex hormones in males. J. Pak. Med. Assoc.42 (9): 210\_/212.

McCoy, A. W. (1991) The Politics of Heroin: CIA Complicity in the Global Drug Trade. New York: Lawrence Hill Books.

Mechoulam, R. and Lander, N. (1980) Cannabis is possible source of new drug .Pharm Inter:19-21.

Murray, R .K ;Granner ,D.K; Mayes ,P .A .and Rodwell .V .W . (2000). Harper Biochemistry 25<sup>th</sup> ed .Pub. Appelton and Lange U.S.A

Nahas,G.(1975).Marihuana; Toxicity and Tolerance. In Richter, EW; Medical Aspects of Drug Abuse. Harper and Row; Hagerstown.

Nahas, G;Leger,C ;Tocoque, B and Hollenger,H.(1981).The kinetic of Cannabinoid distribution and storage with special reference to brain and testis . J. Clin .Pharmacol .21:2085-2145.

Ohlsson, A; Lindgren, J.E; Wahlen,A ; Agrurell,S;Hollister,L.E. and Gillespie ,H .K.(1982).Single dose kinetic of deuterium labeled 9-THC in heavy and light Cannabis users .Biomed.Mass Spectrum.9:6-10.

Patton, W .D .and Pretwee, R .G. (1972) .Effects of Cannabis and certain of its constituents on Pentobarbitone sleeping time and Phenazone metabolism .Brit .J .Pharmacol . 44 :250 .

Permutt , M. A ;Goodwin ,D .W. Schwin , R. and Hillsy, Y.(1976).The effect of marijuana on carbohydrate metabolism .Am .J. Psychiatry 133(2) :220-4 .

Pearce, C.Y; and Cox, J.C. (1980).Heroin , Hyperkalemia; Lancet 2:923

Reynolds, E.F. (1982).Martindale; The Extra Pharmacopoeia. The Pharmaceutical Press; London. 28th Edit.

Sachindra, P.N.(1977) ."Marijuana" Drug Abuse clinical and Basic Aspect. The C.V.mosby Company; Saint Louis 148-173.

Sanz ,R .P ; Vicente ,C . and Repetta , M .(1985). Alteration of glucose metabolism in liver by acute administration of Cannabis . Bull Nac .;37 (4) : 13-15 .

Sawka, M.N; Young, A.J, Francesconi, R.P; Muza, S.R; Pandolf, K.B.(1985).Thermoregulatory and blood responses during exercise at graded hypohydration levels. J Appl. Physiol.59:1394-401.

Schwartz, R.H. (1987). Marijuana; an overview. Pediatric Clinics of North America Vol. 34.

Sheweita ,S.A . (2003) .Heroin changes the expression of cytochrome P450 2E1 .J. Toxo. 191 133;142 140.

Small,E.and Cronquist.,A. (1976).A practical and natural taxonomy for Cannabis; Taxon.25 :405-435.

Steve ,O.Y; Tim ,T.A. (1994 ) Comparisons human and dog (Ret). Seattle Police Department.

Trinder,P . (1969).Ann .Clin .Biochem .6, 24

United Nation International Drug Control Programme (UNDCP); (1997). World Drug Report. New York, Oxford University Press.

Varma , J.K; Malhotra, A.K; Dang , R . (1988). Cannabis and cognitive functions; a prospective study. Drug Alcohol Depend 21(2): 147-152.

Van Sickle,F. (1969). Cannabis properties .Micrograms ;Vol .2,2.

Valbuena, B. A. (1987). Toxicomanias. Ed. Salvat; Madrid.

Wall, M.E. and Perez-Reyes,M.(1981).The metabolism of tetrahydrocannabinol and related cannabinoids in man .J.Clin.Pharmacol. 21 :1785-1895.

Washton, A.M ; Resnick, R.B.(1981).Clonidine in opiate withdrawal: review and appraisal of clinical findings. Pharmacotherapy 1(2): 140-6.

William, G .S. (1985).in U.S Air Force Canine Traing Manual : circa p.34 .



Zuckerman, B; Frank , D.A; Hingson, R.(1989). Effects of maternal marijuana and cocaine use on fetal growth. New Engl J Med; 320 (12); 762-768 .